RT&Tag: S2 cells were collected by centrifuging at 300 g for 5 minutes and washed with 1x PBS. Nuclei were then isolated by incubating with NE1 buffer (10 mM HEPES pH7.9, 10 mM KCl, 0.1% Triton X-100, 20% glycerol, 0.5 mM spermidine, Roche Complete Protease Inhibitor Cocktail, 1 U/µL of RNasin Ribonuclease Inhibitor (Promega)) for 10 minutes on ice. The nuclei were then centrifuged at 500 g for 8 minutes and resuspended in Wash Buffer (20 mM HEPES pH7.5, 150 mM NaCl, 0.5 mM spermidine, Roche Complete Protease Inhibitor Cocktail, 1 U/µL of RNasin Ribonuclease Inhibitor (Promega)). Afterwards, 100,000 S2 nuclei were bound to 5 µL of ConA beads (Bangs Laboratories) for 10 minutes at room temperature. Prior to binding, ConA beads were activated via 2 washes with Binding Buffer (10 mM HEPES pH7.9, 10 mM KCl, 1 mM $CaCl_2$, 1 mM $MnCl_2$). Subsequently, nuclei were incubated with primary antibody diluted 1:100 in Antibody Buffer (20 mM HEPES pH7.5, 150 mM NaCl, 0.5 mM spermidine, Roche Complete Protease Inhibitor Cocktail, 2mM EDTA, 0.1% BSA and 1 U/µL RNasin Ribonuclease Inhibitor) at 4℃ overnight. Afterwards, nuclei were incubated with streptavidin conjugated secondary antibody diluted 1:100 in Wash Buffer (20 mM HEPES pH7.5, 150 mM NaCl, 0.5 mM spermidine, Roche Complete Protease Inhibitor Cocktail) for 45 minutes at RT. Excess antibodies were removed via x2 washes with Wash Buffer. Next, nuclei were incubated with 0.2 mM biotinylated oligo(dT)-ME-B in Wash Buffer for 20 minutes at RT. Excess biotinylated oligo(dT)-ME-B was removed via 2x washes with Wash Buffer. Nuclei were then incubated with ME-A loaded pA-Tn5 diluted 1:200 in 300 Wash Buffer (20 mM HEPES pH7.5, 300 mM NaCl, 0.5 mM spermidine, Roche Complete Protease Inhibitor Cocktail, and 1 U/µL RNasin Ribonuclease Inhibitor) for 1 hour at RT. Excess pA-Tn5 was removed via 3x washes with 300 Wash Buffer. Simultaneous reverse transcription and tagmentation were then performed by resuspending nuclei in $MgCl_2$ containing Reverse Transcription buffer (1x Maxima RT Buffer which contains 50 mM Tris-HCl pH 8.3, 75 mM KCl, 3 mM $MgCl_2$, 10 mM DTT along with, 0.5 mM dNTPs, 10 U/µL of Maxima H Minus Reverse Transcriptase, and 1 U/µL of RNasin Ribonuclease Inhibitor) for 2 hours at 37℃. The nuclei were then washed with 10 mM TAPS and pA-Tn5 was stripped off by resuspending nuclei in 5 µL of Stripping Buffer (10 mM TAPS with 0.1% SDS) and incubating for 1 hour at 58℃. Sequencing libraries were then amplified. The nuclei suspension was mixed with 15 µL of 0.67% Triton X-100, 2 µL of 10 mM i7 primer, 2 µL of 10 mM i5 primer and 25 µL of 2x NEBNext Master Mix (NEB). The following PCR conditions were used: 1) 58℃ for 5 minutes, 2) 72℃ for 5 minutes, 3) 98℃ for 30 seconds, 4) 98℃ for 10 seconds, 5) 60℃ for 15 seconds, 6) Repeat steps 4-5 13 times, 7) 72℃ for 2 minutes, 8) Hold at 4℃. Sequencing libraries were then purified using 0.8x HighPrep PCR Cleanup System (MagBio) beads as per manufacturer's instructions. Sequencing was performed using single-end 50bp sequencing reads.